by any given ratio depends also on the absolute concentrations.

4. The decrease in the rate of accumulation of internal free glutamic acid in these cases is accompanied by the formation of combined glutamate in the medium. Chromatographic examination shows that peptide formation has occurred. In the three cases, the extracellular peptides have been hydrolysed and then give glutamic acid and (i) cysteine, (ii) either alanine or alanine and glycine (two peptides), (iii) glycine; a polyglycine is also formed in (iii), but its formation is independent of the presence of glutamic acid.

5. The presence of glucose is essential for the synthesis of these extracellular peptides.

One of the authors (M.B.V.H.) is indebted to the British Council for a scholarship, during the tenure of which part of this work was carried out. Both authors wish to express their thanks to Dr F. A. Isherwood and to the Director of the Low Temperature Research Station for facilities and advice on chromatographic procedures, and to Dr F. Sanger for the gift of fluorodinitrobenzene and assistance in its use.

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Studies in Vitamin A

16. PREPARATION OF NEOVITAMIN A ESTERS AND NEORETINENE$_1$

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(*Received* 7 *February* 1951)

From the time that the structure of vitamin A was established the possibility had to be admitted that fish-liver oils might contain isomers other than the all-*trans* form, although steric considerations might restrict the number. The most convincing evidence for the existence of *cis-trans* isomers depends: (*a*) on the low yield of crystalline all-*trans*-vitamin A from solutions of the richest concentrates, and (*b*) on the evidence of Robeson & Baxter (1945) that some of the vitamin A in oils and concentrates is slow to react with maleic anhydride. The portion which reacts readily is the all-*trans* form which also crystallizes first. The remainder is neovitamin A, a *cis*-isomer in which only the double bond nearest to the $-CH_2OH$ or $-CH_2OCOR$ group possesses the *cis* configuration. Robeson & Baxter obtained crystalline derivatives of neovitamin A differing from the corresponding derivatives of all-*trans*-vitamin A. They also devised an analytical procedure for determining neovitamin A in fish-liver oils. Their results and those of Meunier & Jouanneteau (1948) indicate that neovitamin A makes up a substantial but variable proportion of the total vitamin A in liver oils. The existence of neovitamin A makes it desirable to know whether the biological activity of fish-liver oils varies with the proportion of the total vitamin A present in the neo form. It is not easy to establish quantitatively a difference between the biological potency of all-*trans*- and neo-vitamin A. Robeson & Baxter failed to find any significant difference, but their experiments may not have been on a scale sufficiently large to establish a small difference.

The existence of neovitamin A also makes it necessary to compare very closely the ultraviolet absorption shown by all-*trans*- and neo-vitamin A. The precise form of the absorption curve is important in the analysis of liver oils, when, as is the rule, irrelevant absorption needs to be allowed for. The simpler procedures for 'correcting' for irrelevant absorption are based on a reference curve for pure all-*trans*-vitamin A and if the vitamin A present in

an oil is spectroscopically heterogeneous, errors may arise depending on the magnitude of differences between the isomers and their relative proportions. In general, *cis-trans* isomerism gives rise to appreciable differences in selective absorption, but such differences cannot be predicted accurately; they need to be determined by experiment. In the case of vitamin A the differences must be slight because there is strong evidence of proportionality between biological activity and intensity of absorption at 328 mμ. for a great range of fish-liver oils; the fiducial limits for biological assays leave room, however, for second order effects which cannot be neglected. It has therefore become important to know how closely the spectrum of neovitamin A in the form of natural esters agrees with that of similar esters of all-*trans*-vitamin A. The original preparation by Robeson & Baxter (1945) depended upon obtaining a very rich concentrate of vitamin A from which the all-*trans* free vitamin A could be crystallized (ethyl formate). The residue after removing the solvent was chromatographed on sodium aluminium silicate. One fraction ($E^{1\%}_{1\,cm.}$, 1410) contained neovitamin A and all-*trans*-vitamin A in the proportions 88 : 12. This material yielded a *p*-phenylazobenzoate which, on recrystallization and hydrolysis, gave crystallizable neovitamin A. The figures in Table 1 enable the two isomeric forms to be compared.

Table 1. *Comparison between all-*trans*-vitamin A and neovitamin A alcohols*

	All-*trans*-vitamin A	Neovitamin A
Melting point ...	62–64°	58–60°
$\lambda_{max.}$ (mμ.) (*iso*propanol)	325	328
$E^{1\%}_{1\,cm.}$ at $\lambda_{max.}$ (*iso*propanol)	1835	1650–1700
Infrared spectra—almost identical		
Biological activity—almost identical		
Anhydro formation—occurs readily, occurs less readily		

According to the maleic anhydride procedure (Robeson & Baxter, 1947; Meunier & Jouanneteau, 1948) various fish-liver oils contained from 18 to 65% of the total vitamin A in the neo ester form. The need to establish the properties of natural neovitamin A esters is thus very real.

METHODS

General

Materials. Some work was done on specially chosen liver oils, but it was generally more convenient to start from ester concentrates made by the Solexol process in South Africa. The materials showed values for $E^{1\%}_{1\,cm.}$ at 328 mμ. of 120–387 and on the whole had extremely little irrelevant absorption.

Adsorbents. Alumina (Peter Spence and Co., Grade 0) was weakened by addition of water (3–10% (w/w) in different experiments). The watered alumina was used the same day.

Solvents. Light petroleum (A.R., b.p. 40–60°) was used in chromatography without purification, but for the more crucial spectrophotometric experiments specially purified material was used. Benzene was used as A.R. quality solvent.

Maleic anhydride (m.p. 50–51°) was purified by sublimation or recrystallized from benzene.

Antimony trichloride reagent. A saturated solution of anhydrous $SbCl_3$ in ethanol-free $CHCl_3$ was used and a little (approx. 1%) acetic anhydride was added just before use.

Spectrophotometry. A Hilger-Nutting visual spectrophotometer was used for the colour test and a Beckman spectrophotometer for the ultraviolet absorption measurements.

Methods of separation

The aim was first to prepare an enriched vitamin concentrate (irrespective of its neovitamin A content) by chromatography on weakened alumina. Little difficulty was experienced in obtaining material of $E^{1\%}_{1\,cm.}$ (328 mμ.) = 600–800. Afterwards, repeated chromatographic separations, depending on the fact that neovitamin A esters are slightly less strongly adsorbed than the all-*trans* esters, permitted gradual enrichment in the neo form. This was at the expense of yield, but it was not necessary from our point of view to obtain large quantities.

Method of analysis for neovitamin A

The procedure of Robeson & Baxter (1947) was slightly modified. A portion of concentrate was accurately weighed and dissolved in benzene. Suitable dilution gave a solution in benzene containing 200–300 i.u./ml. A 5 ml. portion was pipetted into a 10 ml. volumetric flask and made up to volume with a 10% solution of maleic anhydride in benzene. After shaking, the mixture was kept in the dark at 15° for 16 hr. Exactly 1 ml. was then removed and diluted with benzene for use in the $SbCl_3$ colour test. A measured volume of the untreated solution was also tested in the same way. The blue colour ($\lambda_{max.}$ 620 mμ.) was weaker in the solution containing maleic anhydride.

The percentage of the total vitamin A existing as the neo form is given by

$$\frac{R - R_1}{R_2 - R_1} \times 100,$$

where R = percentage recovery of vitamin A in the sample, R_1 = percentage recovery of vitamin A using a pure all-*trans* ester, and R_2 = percentage recovery of neovitamin A ester in a sample of ester free from all-*trans* ester, the criterion of recovery being the appearance of the 620 mμ. maximum in the colour test. The values of R_1 and R_2 are given in Table 2.

Table 2. *Reactivity with* $SbCl_3$ *shown by all-*trans*-vitamin A ester and neovitamin A ester, before and after treatment with maleic anhydride*

(Details of the experimental conditions are given in the text.)

	Relative values		
Material	$E^{1\%}_{1\,cm.}$ at 620 mμ. (no maleic anhydride)	$E^{1\%}_{1\,cm.}$ at 620 mμ. (after maleic anhydride)	Recovery (%)
All-*trans* ester	1·00	0·192	19·2
Neo ester	1·00	0·96	96·0

RESULTS

*Preparation of natural neovitamin A (esters)**

Preliminary chromatographic fractionation. An ester concentrate (1·3 g., *E*, 128) was adsorbed on a column (12 × 1·75 cm.) of weakened alumina (5% water). The chromatogram was developed with light petroleum without suction. The main fraction (0·43 g., *E*, 356) was retained and the residue (0·83 g., *E*, 6) discarded. A second adsorption on a fresh column gave a light petroleum fraction (0·22 g., *E*, 585) and an ethanol eluate (0·165 g., *E*, 3) which was discarded. A third adsorption on a fresh column gave a small anhydrovitamin A fraction which was rejected; the next fraction 3*B* (0·035 g.; *E*, 449) was followed by 3*C* (0·08 g., *E*, 665) and 3*D* (0·04 g., *E*, 757) and 3*E* (0·025 g., *E*, 578). The vitamin of the original concentrate contained 23% neovitamin and 77% all-*trans*-vitamin A. The proportions in 3*B*, 3*C* and 3*D* were 74·5, 26·4 and 7·8% neovitamin A respectively.

The experiment shows the rapid rise in total vitamin A on chromatography. It also shows that neovitamin A is slightly less strongly adsorbed than the all-*trans* form.

Fractionation 1. An enriched concentrate (9·2 g., *E*, 403) containing 15% of its vitamin A as the neo esters was passed through a column (13 × 3 cm.) of weakened alumina (7·5% water). The main fraction (7·7 g., *E*, 527) was passed through a second column (19 × 3 cm.) of weakened alumina (5% water). Two fractions 2*B* (2 g., *E*, 592) and 2*C* (1·75 g., *E*, 761) were retained. These were combined and put through a third column (18 × 1·75 cm., 5% water) and fractions 3*A* (2 g., *E*, 633), 3*B* (0·33 g., *E*, 580) and 3*C* (1·13 g., *E*, 633) were obtained. 3*A* and 3*B* were combined and put through another column yielding 4*A* (0·17 g., *E*, 415) and 4*B* (0·2 g., *E*, 688). Fractions 4*A* and 4*B* contained neovitamin A to the extent of 60 and 40% respectively. The experiment was abandoned at this stage because the degree of enrichment in neovitamin A, though substantial, was not good enough.

Fractionation 2. An ester concentrate (3·3 g., 40% vitamin A esters) gave fractions 1*B* (1·6 g., *E*, 650) and 2*B* (0·73 g., *E*, 781) very rich in total esters. From fraction 3*A* (0·26 g., *E*, 644) fraction 4*B* (0·03 g., *E*, 633) was obtained containing 80% of its vitamin in the neo form. Fraction 3*B* (0·26 g.) gave a first fraction (0·21 g., *E*, 828) which, although made up of about 90% vitamin A esters, only contained 23% in the neo form.

This experiment shows that it is distinctly easier to concentrate the vitamin A esters than to effect a complete separation of the *cis-trans* isomers. The neo percentage was, however, raised to 80 although the yield was small.

Fractionation 3. Two ester concentrates (13 g., *E*, 269; neovitamin 29% and 23·4 g., *E*, 133; neovitamin 24%) were separately chromatographed on weakened alumina (3% water). From them a combined fraction (14·1 g., *E*, 480) was obtained and used as starting point for further work. It gave 1*A* (6·05 g.), 1*B* (1·36 g., *E*, 884) and 1*C* (0·34 g., *E*, 762), 1*B* containing little except mixed vitamin A esters. 1*A*, 1*B* and 1*C* combined gave 2*A* (7·7 g., *E*, 540), which on a fresh column gave 3*A*, 3*B* and 3*C* all with *E*, 540.

* $E^{1\%}_{1\,cm.}$ (325–328 mμ.) is abbreviated below to *E*.

From 3*A* were obtained fractions 4*B* (0·14 g., *E*, 328) and 4*C* (0·5 g., *E*, 602) with 76 and 32% neovitamin A respectively. From 3*A* fractions containing 88 and 42% neo ester were obtained. From 3*C* the best fraction contained 42% neo ester. Combining the 42 and 46% fractions yielded a 70% fraction on further chromatography. The 88 and 70% fractions were combined and put through a further column to yield 6*A* (0·15 g., *E*, 345; 95% neo) and 6*B* (0·105 g., *E*, 460; 72% neo esters).

Fractionation 4. This made use of an enriched concentrate (2·64 g., *E*, 300; 24% neo esters); preliminary fractionations yielded a retained fraction (0·9 g., *E*, 510) from which 1*D* (0·2 g., *E*, 928) was obtained. This on re-adsorption gave a fraction (0·14 g., *E*, 928 with 5% neo ester). Fraction 1*B* in turn gave 2*B* (0·18 g., *E*, 576; 78% neo ester) which in its turn led to 3*B* (0·02 g., 90% neo ester) and 3*C* (0·1 g., *E*, 766; 80% neo ester). It still proved impossible to obtain the neo ester entirely free from the all-*trans* form.

Fractionation 5. This started from 77 g. of ester concentrate (*E*, 279; 23% neo ester). This material was subjected to an elaborate sequence of chromatographic separations. The earliest (least adsorbed) fractions were discarded whenever they showed the anhydrovitamin A bands, however weakly. Many very rich fractions were discarded because the neovitamin A content was less than 35% of the total. By repeatedly combining fractions enriched in neovitamin A esters relatively to all-*trans* esters two fractions were finally retained (0·75 g., *E*, 317; 91% neo ester; 0·51 g., *E*, 382; 86% neo ester). It seems very difficult to accumulate the neo esters without diluting the product with glycerides. The two fractions combined gave in a final fractionation (i), (ii) and (iii). (i) 0·57 g., *E*, 282; 95% neo esters; (ii) 0·34 g., *E*, 416; 90% neo esters; (iii) 0·05 g., *E*, 753; 89% neo esters.

By combining various specimens of small fractions the best neovitamin A ester preparation showed *E*, 810 and < 5% all-*trans* ester.

Absorption curves. The ultraviolet absorption spectra of the best neovitamin A ester fractions have been carefully measured in various solvents. The results are best displayed on a basis of $E_{max.} = 1·00$ and compared with the corresponding curves for all-*trans*-vitamin A acetate (Figs. 1–3). (The curves for the all-*trans*-acetate were measured by Cama, Collins & Morton, 1951.)

The natural neo esters showed relatively to the all-*trans* ester higher absorption from 220 to 280 mμ. and from 330 to 390 mμ. There is apparently a *cis*-peak near 250 mμ. In the region 280–330 mμ. the differences between the neo esters and the all-*trans* esters are small, but not negligible (see p. 47).

Neovitamin A alcohol

Preparation. Various enriched fractions were saponified in the usual manner. It was repeatedly noticed that neovitamin A esters are less readily saponified than the all-*trans* esters, but after refluxing (in the presence of quinol) unsaponifiable fractions were obtained which on chromatography gave neovitamin A in the form of an oil *E*, 1600 and $\lambda_{max.}$, 326–327 mμ., in *cyclo*hexane. The

material could not be crystallized. Repeated attempts to utilize the difficult saponification of neovitamin A esters as a means of enrichment were only partially successful, and the formation of artifacts defeated the project.

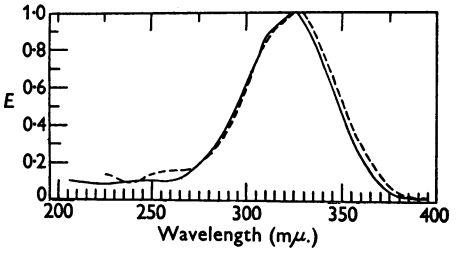

Fig. 1. Absorption curves in light petroleum of: ——, all-*trans*-vitamin A acetate; – – – –, neovitamin A esters (natural). Both curves plotted on a scale where $E_{max.} = 1{\cdot}00$.

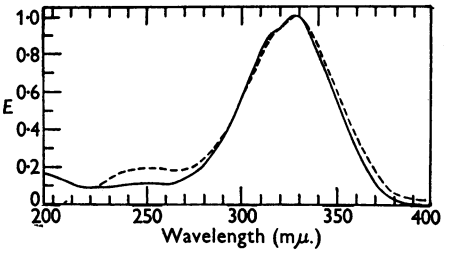

Fig. 2. Absorption curves in *cyclo*hexane of: ——, all-*trans*-vitamin A acetate; – – – –, neovitamin A esters (natural). Both curves plotted on a scale where $E_{max.} = 1{\cdot}00$.

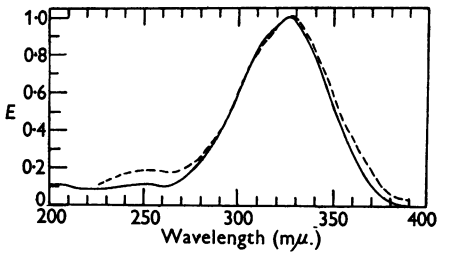

Fig. 3. Absorption curves in ethanol of: ——, all-*trans*-vitamin A acetate; – – – –, neovitamin A esters (natural). Both curves plotted on a scale where $E_{max.} = 1{\cdot}00$.

Absorption curves. Plotted on the basis of $E_{max.}$ 1·00, the curves for neovitamin A alcohol showed higher intensity of absorption from 220 to 290 mμ. and 330 to 390 mμ. as compared with the all-*trans* free vitamin A. The differences, though small, are quite sufficient to influence corrections for irrelevant absorption if neovitamin A predominates over the all-*trans* form.

Irradiation of neovitamin A

The action of light on neovitamin A alcohol or ester resulted at first in a small increase in intensity of absorption, possibly due to formation of some of the all-*trans* isomer.

On exposure to ultraviolet light, ethanolic solutions of vitamin A esters exhibited a sharp increase in green fluorescence. Further irradiation resulted in a decrease, especially if oxygen were not excluded. New inflexions or maxima at 346 and 364 mμ. appeared with a new peak at 275 mμ. and an isosbestic point near 285 mμ. (Sobotka, Kann, Winternitz & Braud, 1944). Similarly, the 328 mμ. maximum of vitamin A acetate in *iso*propanol slowly diminished with the appearance of maxima near 364, 346, 295 and 275 mμ., and if oxygen were excluded the main cause appeared to be light (Bolomey, 1947).

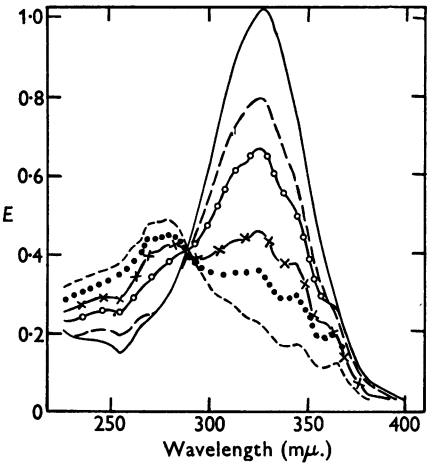

Fig. 4. Absorption curves for irradiated neovitamin A esters ($E^{1\%}_{1\,cm.} = 800$) in ethanol. ——, Zero time; — —, after 3 days in diffuse light; o–o–o, after 9 days in diffuse light; ×–×–×, after 14 days in diffuse light; ....., after 19 days in diffuse light; – – – –, after 28 days in diffuse light.

The effect of light on dilute solutions of vitamin A esters in ethanol has been confirmed in this laboratory using vitamin A concentrates and crystalline vitamin A acetate (unpublished work by Mrs A. W. N. Collins). Exactly similar experiments were carried out using natural neovitamin A esters and the results are shown in Fig. 4.

Oxidation of neovitamin A alcohol to neoretinene$_1$

A neovitamin A ester fraction (0·6 g., E, 342; 95% neo form) was saponified with a trace of added quinol as antioxidant. Chromatography showed that saponification was incomplete and the recovered ester was again treated

with ethanolic KOH under reflux. The total unsaponifiable fraction was oxidized in light petroleum solution by standing over MnO_2 for 12 days in the dark. Purification of the product gave neoretinene$_1$ with $\lambda_{max.}$ 365 mμ. In the colour test $\lambda_{max.}$ occurred at 655 mμ., but moved to 664 mμ. on standing. A preparation on a larger scale showed $\lambda_{max.}$ 365 mμ. (in light petroleum) and 662 mμ. in the colour test.

The differences between retinene$_1$ and neoretinene$_1$ were quite small. As a check, the mother liquors from a first crystallization of a preparation of retinene$_1$ were rechromatographed on alumina, and various portions in repeated operations showed $\lambda_{max.}$ 365 mμ. The neoretinene$_1$ could not be crystallized, but a 2:4-dinitrophenylhydrazone (m.p. 207–208°) and a semicarbazone (m.p. 185°) were obtained.

The absorption maxima for neoretinene$_1$ obtained in various solvents were: light petroleum, 365 mμ.; *cyclo*hexane, 368 mμ.; ethanol, 380 mμ.; chloroform, 385 mμ.; the corresponding figures for retinene$_1$ were 371, 373, 385 and 390 mμ. respectively.

DISCUSSION

The highest intensity of absorption for neovitamin A so far recorded is $E^{1\%}_{1\,cm.}$ (at 328 mμ.) = 1673 (Shantz, 1950). The corresponding figure for all-*trans*-vitamin A alcohol in *iso*propanol is 1835. The difference is not large, but if neovitamin A and all-*trans*-vitamin A are biologically interchangeable, molecule for molecule, the conversion factors for turning spectrophotometric intensities into international units will not be the same. Neither the biological activity nor the molecular extinction coefficient of neovitamin A is as yet sufficiently well established to warrant recommending any complete procedure.

It is evident from Table 3 that so far as the fixation points (Cama *et al.* 1951) are concerned, the displacement is some 2–3 mμ. in the direction of longer wavelengths for neovitamin A as compared with the all-*trans* form. The original fixation points (Morton & Stubbs, 1946) in *cyclo*hexane (313, 328 and 338·5 mμ.) were intermediate between the figures for neo and all-*trans* esters. The differences are least for *cyclo*hexane as solvent and greatest for the free vitamin in *iso*propanol. If the fixation points appropriate to the all-*trans*-vitamin A are used to 'correct' the curve for 100% neovitamin A esters the result would be about 20% too low using *iso*propanol and 14% too low using *cyclo*hexane.

*cyclo*Hexane is thus to be preferred as a solvent in vitamin A determinations. If the light-absorbing solute consists exclusively of the two forms of vitamin A there will be no difficulty in adjusting the correction equations to fit the results of maleic anhydride tests. The problem of highly accurate vitamin A determinations is not, however, a simple one because most oils contain some vitamin A_2 and other products such as kitol, vitamin A epoxide, anhydrovitamin A and other substances contributing irrelevant absorption. It is becoming clear that accuracy will demand a rather elaborate analytical procedure. Extremely careful measurements will first be needed on the purest all-*trans*-vitamin A and on similarly pure neovitamin A. To take advantage of the results it will be necessary to carry out determinations of the all-*trans*-neovitamin A ratio in many liver oils.

Table 3. *Fixation points for neovitamin A*

($\lambda_{max.}$ in mμ. and $\lambda\lambda$ at which $E = 6/7\ E_{max.}$. Corresponding figures for all-*trans* shown in black.)

Solvent	λ_1	$\lambda_{max.}$	λ_2
Natural neovitamin A esters			
Light petroleum	312·5	328	337*
All-*trans*	**310**	**325**	**334·9**†
Δ	2·5	3	2·1
*cyclo*Hexane	314·7	329	339·5*
All-*trans*	**312·5**	**327·5**	**337·7**†
Δ	2·2	1·5	1·8
Ethanol	313·7	328	339*
All-*trans*	**311·5**	**326**	**337**†
Δ	2·2	2	2
Neovitamin A acetate			
*iso*Propanol	314·2	328	339·6‡
All-*trans*	**311·7**	**326**	**336·9**†
Δ	2·5	2	2·7
Neovitamin A alcohol			
*iso*Propanol	312·8	328	337‡
All-*trans*	**310**	**324·5**	**334**†
Δ	2·8	3·5	3

* Determinations made in the present investigation.
† Cama *et al.* (1951).
‡ From curves obtained by an American group (privately communicated).

For many purposes, however, a simple determination of $E^{1\%}_{1\,cm.}$ max. at 326–328 mμ. (in *cyclo*hexane) would probably be as trustworthy a measurement as any when substances other than vitamin A have been eliminated or allowed for. In the present state of knowledge the conversion factor of 1900 would then be appropriate. The reason for not using correction procedures under such circumstances is that at 326–328 mμ. both forms show rather flat maxima.

So far as our experience goes many natural products contain about 25% of the total vitamin A in the neo form. The concentrates were surprisingly uniform in this respect, but much more information is needed before a generalization can be made. If, however, this is confirmed it seems likely that for ester concentrates 'over-correction' need not reach 5%.

The difficulty of saponifying neovitamin A esters

is the only chemical difference we have observed in addition to the difference in speed of reaction with maleic anhydride.

SUMMARY

1. Vitamin A ester concentrates can be enriched by chromatography on weakened alumina until practically free from glycerides, sterol esters and other substances.

2. Repeated chromatographic adsorptions permit the accumulation of neovitamin A esters in the strongly held fractions.

3. Ultraviolet absorption curves for neovitamin A esters closely resemble those of the all-*trans* isomer except that on the long-wave side of 310 mμ. they are displaced by 2–3 mμ.

4. Neovitamin A alcohol yields neoretinene$_1$ on oxidation over manganese dioxide (light petroleum solution). The spectrum of neoretinene$_1$ is slightly displaced in the direction of shorter wavelengths as compared with retinene$_1$.

5. The occurrence of neovitamin A in fish-liver oils may result in over correction in spectroscopic assays if its presence is neglected.

The authors are indebted to the Medical Research Council for financial assistance and to Marine Oil Refiners of Africa Ltd., Simonstown, South Africa, for some of the materials used.

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Studies in Vitamin A

17. SPECTROSCOPIC PROPERTIES OF ALL-*TRANS*-VITAMIN A AND VITAMIN A ACETATE. ANALYSIS OF LIVER OILS

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(*Received* 7 *February* 1951)

The adoption by the World Health Organization (W.H.O.) (1950) of pure crystalline vitamin A acetate as the International Standard substance makes important the specification of criteria of purity. As a result of the widespread use of spectrophotometric methods for determining vitamin A in fish-liver oils and concentrates, spectroscopic properties are specially significant. The necessity for 'correcting' the absorption spectra of many natural products so as to allow for 'irrelevant' absorption adds to the importance of accuracy in the data for pure vitamin A. The advent of synthetic vitamin A as an article of commerce (conceivably contaminated by impurities differing from those found in natural products) raises new analytical problems. The existence of neovitamin A (Robeson & Baxter, 1945), a *cis*-isomer of the better known all-*trans*-vitamin A, and its occurrence in fish-liver oils (Meunier & Jouanneteau, 1948) and some synthetic products (Schwarzkopf, Cahnmann, Lewis, Swidinsky & Wuest, 1949) raises issues which have not been fully studied. The simple correction procedure of Morton & Stubbs (1946, 1948) has been very widely used, sometimes with less caution than is required by the plainly stated assumptions on which it rests.

A simple analytical procedure applicable to all kinds of products is no doubt very desirable, but no such method has yet been described. The substances which can contribute 'irrelevant' absorption include: vitamin A_2, kitol, anhydrovitamins A_1 and A_2, oxidation products, carotenoids and unidentified coloured substances, polyene acids and in synthetic products small amounts of intermediates or artifacts. Other disturbing factors include neovitamin(s) A and the effect of variation in the proportion of esterified and free vitamin A.